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By G. H. Parker

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Biological Laboratories, Harvard University

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I. Introduction

Neurohumors, neurohormones, and chemical activators are general terms applied to a group of substances believed to be concerned in the activation of the nervous system and its appended parts. They are supposed to be the means of transmitting from sense cell to neurone, from one neurone to another, and from neurone to effector cell those communicating functional activities that link together such cells into reflex arcs and other like chains of nervous interrelation. Such substances may be briefly said to be nervous hormones and to partake of the nature of true hormones as originally described by Bayliss and Starling (1902) except in that they are limited to the nervous field. That substances of this kind may exist was first shown, though without recognizing the full import of the matter, by Corona and Moroni in 1898 when they discovered that adrenalin on being injected into a frog would cause it to blanch. Lieben in 1906 independently rediscovered this phenomenon and added to its record the observation that this change in color was due to the contraction of melanophores. In 1918 Redfield pointed out the probable part normally played by adrenalin in the blanching of the lizard *Phrynosoma* since which time this agent has been looked upon as a likely neurohumor in various chromatic animals. Meanwhile the secretion of the intermediate lobe of the pituitary gland, intermedin, was beginning to attract attention as a dispersing neurohumor in amphibians (Smith, 1916a, 1916b; Allen, 1916), a view carried to great fullness by Hogben and his associates (Hogben and Winton, 1922a, 1922b; Hogben, 1924b). These advances were followed by similar discoveries in the chromatic activities of crustaceans. Perkins (1928), following an early observation by Koller (1925), reported in the same year with this worker (1928) that an extract of the eye-stalks of shrimps contained a material, a neurohumor, that when injected into a dark shrimp would induce it to blanch. Thus the neurohumoral hypothesis gained an ever broadening application among chromatic animals.

All these newer observations can be set off collectively against the older and more usual conception of a direct nervous control of chromatophores. Thus these later discoveries led workers like Hogben (1924b) to accept the view that chromatophores in different groups of animals were subject to different kinds of activation, in some instances nervous in others endocrinal or, as it would be designated today, neurohumoral. Hence there came a break with the older traditions, a break which may be set in general at about 1920, and which marks the introduction into this field of the novel agent, the neurohumor, which at least shared the responsibility with pure nerve action as a means of exciting chromatophore responses. The animals to which this interpretation of the excitation of color changes applied were certain crustaceans, particularly shrimps and prawns, and the lower vertebrates, fishes, amphibians, and lizards. The only other extensive group of forms in which color changes are significant is that of the cephalopods which includes such creatures as the octopus, the cuttlefish, and the squid. In these animals the chromatophores, which as a matter of fact are the particular effectors to which this name was originally applied (Sangiovanni, 1819), are much more complex than are those in the crustaceans and the vertebrates. In cephalopods the chromatophores are veritably miniature organs, for they consist each of a cellular sac of colored fluid surrounded by a circlet of innervated, smooth muscle-fibers by which the sac may be drawn out from its spherical form into a broad disc. On the relaxation of the muscle-fibers this sac whose wall is elastic reassumes its spherical shape and thus changes from a broad, circular, colored area into an inconspicuous dot. Plainly such a chromatophore, though serving the same purpose as the similarly named effectors in the crustaceans and the vertebrates, is structurally much more complex than are these which are either individual

cells or at most small groups of cells. The neurohumoral problem in cephalopod chromatophores is that of the relation of nerve-fibers to smooth muscle-cells, the portion of the organ by which the color-sac is operated, whereas that in the crustaceans and the vertebrates has to do with nerve-fibers that impinge at once on color cells whose operations involve streaming protoplasm. The conditions in the crustaceans and the vertebrates, physiologically more primitive perhaps than those in the cephalopods, are what will be considered in the present account and nothing further will be said about cephalopod chromatophores and their complications.

II. Crustacean Neurohumors

The crustaceans that exhibit color changes are found almost exclusively among the larger types, the malacostracans, and range from the isopods to the decapods. The first crustacean in which this activity was clearly observed was the prawn *Hippolyte* (Kröyer, 1842) and the earliest account of the crustacean chromatophore was drawn from the schizopod *Mysis* (Sars, 1867). The experimental study of color changes in crustaceans may be said to have been initiated in 1872 by Pouchet, and the subject was firmly established and greatly extended by Keeble and Gamble (1900–1905) in their classical investigations carried on during the early years of the present century.

A crustacean chromatophore consists usually of a group of closely associated cells or better perhaps a syncitium containing a number of nuclei. Its central mass, however, is often so divided as to suggest separate cells. In its expanded condition it possesses many long branching processes which reach out from its center into the adjacent tissue. In its contracted state these processes are said not to be visible with certainty. On re-expansion, however, the processes reproduce closely their original outlines as can be shown by successive photographs (Perkins, 1928). The central mass of the chromatophore contains a dense accumulation of pigment which in expansion passes out in part into the branched processes. This pigment may be of one or more kinds. In *Crangon* (Koller, 1927, 1928, 1930; Abramowitz, 1937b), *Portunus ordwayi* (Abramowitz, 1935c), *Uca pugilator* (Carlson, 1936) and the like four kinds of pigment are present,—yellow, red, black, and white. Some decapods have only three of these pigments; thus in *Uca pugnax* red is almost lacking, in *Portunus anceps* it is entirely so (Abramowitz, 1937b, 1935c) and in *Palaemonetes vulgaris* (Perkins, 1928; Brown, 1935) and in *Leander adspersus* (Hanström, 1937) black is absent. Two or even three kinds of pigments may be found in the same chromatophore. Thus di- and terchromatic chromatophores may occur, as, for instance, in *Hippolyte* and *Crangon* (Keeble and Gamble, 1900–1905), in *Palaemonetes* (Brown, 1935) and in other decapods. Scattered among these polychromatic chromatophores are also to be found color cells containing only one type of pigment. In *Portunus anceps* (Abramowitz, 1935c) the three pigments, white, black, and yellow, are always each in a separate chromatophore, and the same is true of the four pigments, dark, red, yellow, and white, in *Uca pugilator* (Carlson, 1936). Thus, though these crabs themselves may be said to be polychromatic, their individual chromatophores are monochromatic. The polychromatism which appears to characterize many species of decapods contrasts strongly with the monochromatic condition of the two isopods *Ligia oceanica* (Tait, 1910) and *L. baudiniana* (Kleinholz, 1937). Both these species may be said to possess only melanophores, for the "guanophores" described in *L. baudiniana* appear to be of little real significance.

A monochromatic crustacean such as *Ligia* has only a limited range of color change with dark and pale as its two extremes. In polychromatic forms there may be much wider possibilities; thus *Palaemonetes* may adapt itself with more or less success to backgrounds of white, yellow, red, green, blue, dark gray, and black (Brown, 1935). *Crangon vulgaris* also is capable of extensive adaptation, but lacks the ability to change to green or blue (Koller, 1927). Pouchet (1873) early called attention to a blue pigment in crustaceans which appeared to emanate as a solution from certain chromatophores and to spread in the form of a cloud into the adjacent tissues where it disappeared. This was seen in *Hippolyte* by Gamble and Keeble and has since been identified in *Palaemonetes* (Perkins, 1928), in *Macrobrachium* (Smith, 1930), and in *Portunus* (Abramowitz, 1935c). It is a type of extrachromatophoral pigment as contrasted with the intrachromatophoral colors already alluded to. Both *Crangon* and *Palaemonetes*, as already mentioned, can adapt themselves with more or less success to the color of the environment. *Uca* (Megašur, 1912) on the other hand lacks this ability but shows instead a pronounced diurnal rhythm, being dark by day and pale by night (Abramowitz, 1937a). This type of color change has been known since

the days of Gamble and Keeble who recorded it in *Hippolyte,* an observation recently confirmed by Kleinholz and Welsh (1937). It has also been identified in *Idothea* by Piéron (1913, 1914), in *Leander* by Hanström (1937), and in *Ligia* by Kleinholz (1937). These in brief are some of the more striking phenomena concerned with the color changes of crustaceans, changes which in the opinion of investigators prior to 1925 were supposed to be due to peripheral nerve action. This belief that crustacean chromatophores are directly influenced by peripheral nerves was based upon the well-established fact that after the removal of the eyes from a chromatic species color adaptation with all its manifold complications disappeared. But this evidence, though conclusive for the importance of the eye in these reactions, fell short of showing that such chromatophore responses were dependent directly upon peripheral nerves.

The opinion that such responses in crustaceans were neurogenic in origin was persistently held by the older workers notwithstanding the fact that they were unable by the electric stimulation of nerve tracts in these animals (Pouchet, 1872, 1876; Gamble and Keeble, 1900) and by the cutting of their peripheral nerves and their central nervous organs (Pouchet, 1876; Yung, 1878; Mayer, 1879; Matzdorff, 1883; Gamble and Keeble, 1900; Fröhlich, 1910; Menke, 1911; Degner, 1912) to obtain anything but negative results. Tests on nerves were carried out very fully by Koller (1925, 1927) in *Crangon* and especially by Perkins (1928) in *Palaemonetes* with the result that both investigators were forced to conclude that the central nervous organs and the peripheral nerves in these crustaceans were not concerned with chromatophoral responses. The first suggestion of the way in which crustacean chromatophores were controlled came from an observation by Koller (1925) who noted that when the blood of a dark *Crangon* was injected into a pale one kept in light surroundings the pale individual in ten to twenty minutes became dark. On the other hand blood from a pale *Crangon* when introduced into a dark or a pale one had no effect upon the recipient's color. These conditions led Koller to suspect that the chromatic changes in this shrimp were under the control of internal secretions.

Perkins (1928) who, as already noted, had abandoned the idea that the central and peripheral nervous systems in *Palaemonetes* were concerned with this shrimp's color changes, turned to a study of its body fluids and devised a very simple but effective method of temporarily occluding the passage of blood through its dorsal abdominal blood-vessel. When the flow of blood in this vessel in a dark shrimp was stopped and the animal put on a white background, the whole shrimp blanched except that part of the abdomen which was without circulation. This remained dark, but on restoring its blood supply it rapidly became blanched. When a pale individual with an occluded vessel was transferred to a jar with black walls, the animal darkened as a whole except posterior to the point of the stoppage of blood where it remained pale. On releasing the vessel this posterior portion darkened. These observations led Perkins to assume that an active agent concerned with the color changes in *Palaemonetes* was carried in its blood and he proceeded then to look for an organ in this shrimp by which such a substance might be produced. After much search this was found to be the eye-stalks of the animal. When the eye-stalks of a pale *Palaemonetes* were removed, the shrimp darkened and when several of these stalks were crushed in sea-water and extracted and the extract was injected into a blinded shrimp whose chromatophores in consequence were expanded, these color cells contracted. No color changes were seen when similar injections were made into pale *Palaemonetes,* nor did ordinary sea-water, when introduced into this form, bring about any color alteration in either the pale or dark individuals. From these observations Perkins concluded that the eye-stalks of *Palaemonetes* produce a substance, a neurohumor, that induces a contraction of chromatophores. He was unable, however, to find out the means by which this shrimp brought about an expansion of its color cells. Perkins' discovery of the source of the chromatic neurohumor in *Palaemonetes* was quickly confirmed by Koller (1928) in *Crangon,* and in this shrimp Koller believed further that he had located a rostral organ responsible for the expansion of the chromatophores.

Perkins' announcement that an activator of chromatophores was to be found in the crustacean eye-stalk was soon substantiated in a large number of malacostracans, including the stomatopod *Squilla* and the mysids *Praunus* and *Mysis.*[1]

[1] Active eye-stalk neurohumors have been obtained from representatives of the following genera of brachyuran decapods: *Callinectes* (Perkins and Kropp, 1932; Hanström, 1935, 1937; Carlson, 1936), *Cancer* (Perkins and Kropp, 1932; Kropp and Perkins, 1933; Hanström,

In all these crustaceans an extract of the eye-stalk when injected into another individual of the same or related species induced a color change. The only exceptions to this rule thus far found occur in the anomuran decapods *Hippa* and *Gebia* and in the isopod *Ligia*. Extracts from the eye-stalks of *Hippa* and of *Gebia* were found by Hanström (1935) to be inactive in exciting color changes. The tissue in the heads of these forms and immediately below the base of their eye-stalk was found, however, to induce color changes. Hanström, therefore, concluded that in these two crustaceans the part of animal concerned with the formation of the neurohumor appropriate for color change had shifted from the stalk to a subjacent position, a condition that probably also characterizes *Emerita, Gebiopsis, Calocaris,* and *Callianassa* (Hanström, 1937). This view was confirmed by Carlson (1936). In the isopod *Ligia* as in all other members of its group the eyes are sessile, the stalk being absent. It is not surprising, therefore, that here as in *Hippa* and *Gebia* the tissue of the head yields a chromatophore activator as was first demonstrated by Kleinholz (1937). Thus in one way or another all chromatic crustaceans whose color changes have been investigated in this respect are known to possess neurohumoral organs, though the locations of these organs are not always the same. It is an interesting fact, pointed out by Kropp and Perkins (1933) that some crustaceans such, for instance, as *Homarus, Libinia,* and *Cancer,* which lack obvious integumentary chromatophores, nevertheless possess vigorous eye-stalk neurohumors. This condition was also noted by Hanström (1937) who in discussing the matter was led to conclude that the chromatic neurohumors in these forms may well have other functions than that of regulating color changes. Thus, for instance, they may be concerned with controlling the movement of the retinal pigment, or with the calcium balance in the blood or with other like metabolic activities. It is also to be kept in mind that though the adults of these crustaceans may be devoid of effective color changes, the young may not and that the chromatic neurohumors found in the adults may be merely residues of those whose real importance belongs to an earlier stage.

Since the eye-stalks or adjacent tissues in crustaceans generally produce a chromatic neurohumor, it is natural to inquire what particular organ in or about these stalks is concerned with this function. Koller in 1930 demonstrated that an extract from the cap of the eye-stalk in *Crangon,* namely from that part composed chiefly of retina, was quite inactive as a chromatic agent, but that one from the non-proximal portion of the stalk was fully efficient. These observations were confirmed by Hosoi (1934) in *Penaeus.* By very local cauterization on *Crangon* Koller was led to place this neurohumoral generator near the basement membrane of the retina. Biological tests of different parts of the eye-stalk in *Penaeus* induced Hosoi to conclude that this secretory organ in *Penaeus* was located proximal to the position assigned to it in *Crangon* by Koller, but not so far as the middle of the stalk. More precise localization studies were afterwards carried out by Carlson (1935, 1936) and by Hanström (1935, 1937). Hanström (1934) had pointed out that the crustacean eye-stalk often contained two innervated glandular organs, the X-organ and the blood gland, or as it has subsequently been called, the sinus gland (Hanström, 1937). One or both

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1935, 1937; Carlson, 1936; Abramowitz, 1937), *Libinia* (Perkins and Kropp, 1932; Kropp and Perkins, 1933a; Hanström, 1935; Carlson, 1936; Abramowitz, 1937b), *Carcinus* (Hanström, 1935, 1937; Carlson, 1936; Abramowitz, 1937a), *Ovalipes* (Hanström, 1935, 1937; Carlson, 1936), *Panopaeus* (Hanström, 1935), *Uca* (Carlson, 1935, 1936; Abramowitz, 1936b, 1937b), *Aratus* (Hanström, 1937), *Ocypoda* (Hanström, 1937), *Sesarma* (Hanström, 1937); from representatives of the anomuran genus *Pagurus* (Kropp and Perkins, 1932; Perkins and Kropp, 1933; Hanström, 1935, 1937; Carlson, 1936; Abramowitz, 1937a); from representatives of the macruran genera: *Crangon* (Koller, 1928, 1930; Koller and Meyer, 1930; Perkins and Kropp, 1932; Kropp and Perkins, 1933; Hanström, 1935, 1937; Carlson, 1936; Abramowitz, 1937a 1937b), *Leander* (Koller, 1928; Hanström, 1935, 1937; Kleinholz and Welsh, 1937), *Macrobrachium* (Smith, 1930), *Palaemonetes* (Perkins and Snook, 1931, 1932; Perkins and Kropp, 1932; Brown, 1935; Kropp and Crozier, 1934; Navez and Kropp, 1934; Hanström, 1935, 1937; Carlson, 1936; Abramowitz, 1937a, 1937b), *Homarus* (Perkins and Kropp, 1932; Kropp and Perkins, 1933; Hanström, 1935, 1937; Abramowitz, 1937a), *Pandalus* (Perkins and Kropp, 1932; Kropp and Perkins, 1933), *Palaemon* (Beauvallet and Veil, 1934), *Paratya* (Hosoi, 1934), *Penaeus* (Hosoi, 1934; Hanström, 1937), *Cambarus* (Hanström, 1935, 1937; Carlson, 1936), *Astacus* (Carlson, 1936), *Acanthephyra* (Hanström, 1937), *Hippolyte* (Kleinholz and Welsh, 1937); from representatives of the stomatopod genus *Squilla* (Hanström, 1937); and from representatives of the schizopod genera *Mysis* (Koller and Meyer, 1930; Perkins and Kropp, 1932; Kropp and Perkins, 1933) and *Praunus* (Koller and Meyer, 1930; Hanström, 1937).

of these might be the producer of the chromatic neurohumors. Carlson (1935) cut the long eye-stalks of *Uca* into three equal parts. Of these only the middle one yielded an extract that was chromatically active. If the distal parts of the eye-stalk were cut from a living crab, the animal would maintain its original dark color. If now the middle part was removed, the crab would grow pale as it would after the removal of the entire stalk. Carlson, therefore, concluded that the seat of origin of the chromatic neurohumor in this crab was in the middle third of its eye-stalk. A histological examination of the eye-stalk of *Uca* showed no evidence of an X-organ there, but demonstrated the presence of a well-developed sinus gland in the middle section of the stalk. Hence Carlson (1936) concluded that the sinus gland is in all probability the source of the neurohumor. This conclusion was accepted by Hanström (1935) who also showed that the position of the X-organ and the sinus gland in *Pagurus* likewise pointed to the latter as the source of the chromatic neurohumor. Hanström (1937) further demonstrated that in those crustaceans in which the eye-stalks were more or less reduced, but in which the tissue below them yielded a chromatic neurohumor, as for instance in *Hippa* and *Gebia*, a sinus gland was not to be found in the stalk, but was to be located in the subjacent region. It is, therefore, now generally admitted that the sinus gland, a structure discovered by Hanström, is the source and probably the all important source of the crustacean chromatic neurohumor. Hanström (1935, 1937), however, has called attention to the current belief that there may be several such neurohumors and that it will be well not to dismiss the X-organ completely from the category of possible chromatic glands till more complete evidence is at hand.

Suggestions of other possible sources for chromatic neurohumors in crustaceans have not been wanting. Extracts of the ventral nerve cord of *Palaemonetes* (Brown, 1933) and of *Penaeus* (Hosoi, 1934) were found to be active as excitants of color changes and Hosoi found this also to be true of extracts of the male genitalia, the stomach, and the muscles of *Penaeus*, but not of the heart. All these positive reactions, however, may be due, as Kleinholz remarked (1936a), to traces of the hormone present in the blood and held within the various organs tested. Such records as evidence of additional sources of chromatic neurohumors can scarcely be accepted until this whole subject has been thoroughly reinvestigated.

The question of a rostral organ concerned with the elicitation of the dark phase in crustaceans and particularly in *Crangon* (Koller, 1928, 1930) is still a matter of debate. This organ was described by Koller as situated in the dorsal part of the cephalothorax of *Crangon vulgaris* directly behind the rostral spine. According to Koller's most recent statements (1938) evidence from four sources favor the belief in this part of the shrimp as the seat of an activating organ for the dark phase. If a pale *Crangon* on a white background is fed with pieces of different organs from a dark individual such, for instance, as pieces of stomach, liver, gonads, muscles, eyes, and rostral tissue, the last named is the only one that will excite an expansion of the dark pigment. Extracts of the rostral region when injected into a pale *Crangon* will induce darkening in some twenty minutes, while control injections are without effect. Destruction of the rostral region leaves the shrimp unable to darken. Finally pressure on the rostral region of a pale shrimp is followed in four minutes or so by darkening. These observations confirm Koller in the belief that the rostral region of this shrimp contains tissue active in producing a darkening neurohumor. Other investigators have not been able to demonstrate a dispersing chromatic organ in the rostral regions of the crustaceans studied by them. Thus Perkins and Snook (1931) state that all their attempts to expand the chromatophores of *Palaemonetes* by injecting rostral extracts from this shrimp met with failure and a similar report is made by Brown (1935) who also worked on *Palaemonetes*. Kropp and Perkins (1933) likewise obtained negative results from their tests of the American shrimp *Crangon boreas*. In no instance could they obtain a chromatophore-expanding action following an injection of rostral extract into a pale *Crangon* though care was taken to prepare the material from the region designated by Koller and in the manner prescribed by him. Beauvallet and Veil (1934) working on *Palaemon* found that rostral extracts from this shrimp would turn pale individuals dark. This change, however, was only an occasional happening and occurred only when very concentrated solutions were used. Since Koller's results have not been confirmed on *Palaemonetes* or on *Crangon boreas*, it may be that the possession of a rostral organ is, so far as present knowledge is concerned, a peculiarity of *Crangon vulgaris*. The solution of this difficulty must await further investigation.

It must be evident from what has been stated

that of the various suggested sources for the chromatic neurohumors in crustaceans the only certain one is the sinus gland in or near the eyestalk. How many neurohumors are produced by this or other possible glands and what they are like chemically are questions which for the present are beyond answer. At best only approximate intimations can be offered. The first neurohumoral extract to be identified was that obtained by Perkins (1928) from the eye-stalks of *Palaemonetes*. This had the general property of blanching *Palaemonetes* by concentrating its dark chromatophoral pigment. Perkins was unable to find any other neurohumor in this crustacean. Koller (1928), as just stated, believed that *Crangon* possessed in addition to this a rostral-dispersing humor, but the uncertainty of this view must be evident. Admitting its correctness, however, *Crangon* could then be said to possess at least two neurohumors. To these Koller gave the names of expantin and contractin. He also advanced reasons for assuming another neurohumor in *Crangon* for the dispersion of yellow. He thus raised the total number of chromatic neurohumors in this shrimp to three (Koller, 1938). Brown (1935) who studied the responses to different backgrounds of the four pigments in the chromatophores of *Palaemonetes* adduced evidence to show that these pigments were capable of complete independence in their activities. To determine this it was necessary to observe simultaneously two pigments as the shrimp was treated experimentally and to find situations in which these pigments were able side by side in the same animal to show all possible combinations of behavior in migration. A sample of this kind of treatment is given in Table I which is taken from Brown paper of 1935.

As an example of this kind of treatment the independence of the responses of the red and the yellow pigments may be considered. This is shown in column A of the table where are indicated the various background responses which when taken collectively demonstrate the independence of the red and yellow colors. Thus on white both these are concentrated, on red the red pigment is concentrated, and the yellow dispersed, on dark gray red is dispersed and yellow concentrated, and on black both are dispersed. By evidence of this kind Brown was led to declare that all four pigments in *Palaemonetes* were capable of independent response and to assert that no fewer than four neurohumors must be assumed for the various chromatic changes in this shrimp. It was Brown's opinion that in these changes concentration was a humoral response and dispersion an activity due to the chromatophore itself without aid from a neurohumor. Such views as those put forward by Koller and by Brown in which more than one neurohumor is involved have been brought together by Abramowitz (1937b) under the general head of the multiple theory of chromatophoral action as contrasted with what he has called the unitary theory of these activities in which, as will be explained presently, only one neurohumor is involved.

It was established by Perkins (1928) in the early period of the study of crustacean neurohu-

TABLE I

Tabular representation of the condition of the four pigments in animals upon colored backgrounds. In vertical column A are checked those backgrounds which, taken together illustrate that the red and yellow pigments behave independently of one another. Column B does the same for the combination of red and white pigments; C for the yellow and white; D for the yellow and blue; E for the red and blue; and F for the blue and white. (Brown, 1935)

Background color	Condition of red pigment	Condition of yellow pigment	Condition of white pigment	Blue network	A	B	C	D	E	F
White	Conc.	Conc.	Disp.	Absent	X	X	X	X	X	X
Green	Conc.	Conc.	Disp.	Present						X
Red (1)	Conc.	Disp.	Disp.	Absent	X		X	X		
Red (2)	Disp.	Disp.	Disp.	Absent		X			X	
Blue	Conc.	Conc.	Conc.	Present		X	X	X	X	
Dark gray	Disp.	Conc.	Conc.	Present	X					
Black	Disp.	Disp.	Conc.	Present	X	X	X	X	X	X
Darkness	.....	.....	Conc.	Absent						X

mors that when the eye-stalks are cut off from *Palaemonetes*, the shrimp darkens. It was also shown by Perkins that when into such a darkened individual an extract of its eye-stalk is injected, the shrimp will blanch temporarily. Carlson (1935) was the first to point out that the reverse of this was true of the crab *Uca*. If its eye-stalks are removed, it blanches instead of darkens, as in fact Megušar (1912) had long ago shown. If now an extract of its eye-stalks is injected into a stalkless and consequently pale *Uca*, the latter darkens. Thus *Uca* reacts both to the loss of its eye-stalks and to their extract in the opposite way that *Palaemonetes* does to a similar loss and to its own eye-stalk extract. These remarkable conditions were fully discussed and further illustrated in 1936 by Carlson who also pointed out the following additional peculiarity. When the eye-stalk extract from *Uca* is injected into another *Uca*, stalkless and consequently pale, the recipient, as already mentioned, darkens. When such an *Uca* extract is injected into a stalkless and consequently dark *Palaemonetes*, this shrimp blanches. Here then the same extract which darkens a stalkless *Uca* blanches a stalkless *Palaemonetes*. These very interesting observations were fully confirmed and somewhat extended by Abramowitz (1937b) who was led to remark that such results "show that an extract of the eye-stalks, regardless of its source, produces the same qualitative chromatophoral responses in a particular species as those induced by injection of the species' own stalk extract. This obviously means that either there is present in the eye-stalks of every crustacean investigated one pigmentary hormone whose effects are determined by the particular chromatophoral organization of the species into which it is injected, or that each crustacean contains in its eye-stalks a veritable array of hormones, one for but one of the two phases of a particular pigment, so that depending upon the species injected, a certain pigment may be now contracted, now expanded." These new discoveries appear to brush aside at once, as Abramowitz remarks, Koller's proposed types of neurohumors, expantin and contractin, for a single extract, and presumably a single neurohumor, acts on one crustacean as a dispersing agent and on another as a concentrating one. These observations also set the whole question of the number of chromatic neurohumors in crustaceans in a new light and call for a radical reinvestigation of the whole field.

Abramowitz (1937b) who has discussed this question with much fullness is of opinion that either there are numerous specialized hormones acting upon the body of chromatophores (multiple theory) or one hormone to which there are different types of chromatic response in accordance with chromatophoral organization (unitary theory). According to Abramowitz the present body of observations in this field may be interpreted on the basis of either conception. He states, however, that if Brown's tabulated observations are correct, it "is almost inescapable that there must be more than one hormone." And he adds that "future studies may well reveal that many or at least more than one hormone are secreted by the eye-stalk" though, as he remarks, this view is not supported by present evidence.

From what is now known of the reactions of chromatophores in crustaceans, it would be rash indeed to attempt any prediction as to the number of neurohumors. Their one certain source, the sinus gland, is so simple in structure that it seems to favor the unitary theory, but there are other possible glands, as Hanström has remarked, that must not be forgotten. Furthermore, the sinus gland may not be so simple in organization as at first sight it appears to be. It is also possible that a single neurohumor may act differently at different concentration as suggested by Przibram (1932) and thus play more than one part. That chromatophores are different in organization, as has been insisted upon by Abramowitz (1937 b), is beyond cavil. Even within a single category, such for instance as melanophores, diversity reigns. Adrenalin, which is the only chromatic neurohumor whose chemical purity can be assured is well known to disperse pigment in retinal melanophores as, for instance, in the frog, and to concentrate it in the melanophores of this animal's skin. Even in the integument the menalophores in one spot react to the same reagent differently from what they do in another. It must also be kept in mind that the individual chromatophore probably changes in its receptiveness to exciting agents from moment to moment. A melanophore after dispersing its pigment may not be the same as it was when its pigment was concentrated. The mere operations of concentrating and dispersing pigments may change the cell. Hence it is not inconceivable that the same agent may find a different type of reception in a color-cell with expanded pigment from what it does in the same cell with contracted pigment. These suggestions favor for the most part the unitary theory, but the opposing view, the multiple

theory, is not without support. First of all it must not be forgotten that the multiple theory does not necessarily require such a large array of neurohumors as has been suggested, for instance, by Abramowitz (1937b). In fact it is possible that the four neurohumors hypothesized by Brown for *Palaemonetes* may not all be needed. It is conceivable that by appropriate combinations fewer elementary neurohumors may suffice. Thus if in a purely formal way three basic neurohumors A, B, and C are assumed, there may be made from these the additional combinations AB, AC, BC, and ABC which with the original A, B, and C give a total of seven agents. Such a condition could obtain, of course, only provided each combination is in some sense a combined product and not merely elements mixed. Relations of such a kind, however, would be by no means surprising when the complexity of organic interactions is kept in mind. In this way a final complexity of agents might rest on a relatively simple base and the so-called unitary and multiple theories might thus be brought more nearly together. These two views as already formulated are perhaps somewhat too rigid and inelastic to meet the exigencies of a situation whose difficulties call for experimental solution. They possess an obviousness and a clearness which in a measure carries with it their own fate, for the unitary theory must be discarded on the discovery of two neurohumors. The real question involved is not whether there is one or more neurohumors, but what the exact number is. However, till a chemical clarification of the state of the eye-stalk extract is made, it is futile, as Abramowitz remarks, to speculate on the exact number of its neurohumors. Most workers have placed this approximately at several (Hanström, 1938).

If little can be affirmed as to the number of crustacean neurohumors still less can be said of their chemical nature. That the neurohumor in *Palaemonetes* is soluble in water (hydrohumor) was demonstrated by Perkins (1928) in his initial experiments. That it is not destroyed by boiling or drying was recorded by Perkins and Snook (1931), whose observations in this respect have been confirmed by many subsequent workers. In fact it has been shown that boiled extract is often more effective than unboiled (Carlson, 1936). Carlson (1936) found that the neurohumor in *Palaemonetes* would diffuse through a cellophane membrane, that it was not soluble in ether, but could be dissolved in alcohol and that it was not destroyed by being boiled in either weak acid or weak alkali. The eye-stalk neurohumor from *Uca* was stated by Abramowitz (1937a) to be readily soluble in water, not completely soluble in ethanol or methanol, only slightly soluble in acetone, and insoluble in benzine, chloroform, or ether. It does not decompose when boiled with weak acid or alkali for a short time, but does break down completely after being boiled for two hours in weak alkali. It is thermostable and not destroyed by drying. It can be kept in an aqueous solution in a refrigerator for some time without loss of activity, but in water and at room temperature it slowly disappears. Little more can be said of the chemical nature of these bodies; the facts thus far contributed show with reasonable certainty that they are not typical proteins, but their exact chemical nature is at present quite obscure.

From what has been stated in this survey of the activation of crustacean chromatophores, it is clear that the the old view of direct nervous stimulation of these color cells must be completely abandoned. The crustacean eye and its central nervous connections are means of controlling the secretions of the sinus gland and other like structures. Thus far the operation is nervous, but the neurohumor produced in this way is carried by the blood to the distantly located chromatophore which is strictly a hormonal or humoral procedure. This second step in the operation is much the more considerable from the standpoint of space covered and yet it was entirely overlooked by the older workers. Thus the real stimulation of chromatophores in crustaceans is a purely neurohumoral affair carried out by a hydro- or water-soluble humor. It lacks all traces of direct nervous action.

III. Vertebrate Neurohumors

1. *Introduction*

The interpretation of the control of vertebrate chromatophores was in the beginning strictly nervous like that for the color cells in crustaceans. This view of nervous activation was subjected to experimental test and was fully discussed as long ago as 1852 by Brücke in his account of the color changes in the chameleon. It was accepted in detail by Lister in his admirable essay on the chromatic reactions of the common frog published in 1858. It was the main thesis of Pouchet's brilliant memoir on the chromatophores of fishes issued in 1876. The demonstration by histological means of the nerve endings on the melanophores in teleosts made simultane-

ously by Ballowitz (1893) and by Eberth (1893) seemed to be the final link in the chain of evidence in its favor. It permeated completely von Frisch's scholarly contributions to the study of teleost coloration (1910, 1911a, 1911b, 1912a, 1912b). It was the only view admitted by Biedermann in his exhaustive treatment of the color changes in frogs (1892). A survey of the many papers dealing with this field from the standpoint of those just mentioned leaves the impression that the system of vertebrate chromatophores, like that of their muscles, is composed of effectors activated exclusively by nerves.

In most of the later contributions to this subject which favored the neurogenic conception of chromatophore activation, there was no consideration whatever of a growing opinion that the activation of chromatophores might be due, in part at least, to the special hormones known as neurohumors rather than to nerves. This opinion was first suggested by the work of certain experimental embryologists. Some two decades ago Smith (1916a, 1916b) and Allen (1916, 1917) showed independently that frog tadpoles, which had been deprived previously of the rudiments of their pituitary glands, became pale and remained so indefinitely. The importance of the pituitary gland in this respect was confirmed subsequently by Atwell (1921), Giusti and Houssay (1921), Swingle (1921), Krogh (1922) and a number of others, but particularly by Hogben and his associates who in a series of highly original contributions beginning in 1922 carried the whole subject forward with great success. In particular Hogben and Winton (1922a, 1922b, 1922c, 1923) showed that in the common frog cutting or stimulating its sciatic nerve was not followed by color changes in the skin of the leg tested. They confirmed the observations of earlier workers that the loss by the frog of its pituitary gland was invariably followed by blanching which could be temporarily changed to darkening by an injection of extract from the pituitary gland of another frog or of almost any other vertebrate. The darkening of a pale, hypophysectomized frog could also be induced by the implantation into its body of a fresh pituitary gland. Thus Hogben and Winton (1923) were led to conclude that the nervous factor in the control of the color changes of the frog was at best insignificant and that these changes were due primarily to fluctuations in the amount of pituitary secretion liberated within the animal. The momentary coloration of a given frog was in their opinion an index to its own pituitary activity. After a general survey of the field of integumentary color activation in the vertebrates, Hogben (1924b) concluded that there must be striking differences in the chromatophore systems of these animals, for the color cells of fishes and of reptiles appeared to be under nerve control while those of amphibians were activated by hormones. Such a conclusion, though warranted by the facts as then known was not wholly satisfactory from a zoological standpoint, for it would be strange indeed if fishes and reptiles, two groups well separated systematically, should exhibit a common type of chromatophore activation differing fundamentally from that of the intermediate group, the amphibians. It seemed, therefore, desirable to undertake a restudy of the means of excitation of color changes in a number of chromatic vertebrates and thus to ascertain how considerable their differences really were. To this end the common frog, certain dogfishes, *Mustelus* and *Acanthias*, certain teleosts, *Fundulus* and *Ameiurus*, as well as the western lizard *Phrynosoma*, were made the objects of special investigation.

2. *Amphibians*

In the earliest account of the color changes in frogs (Vallisnieri, 1715), these changes, according to van Rynberk (1906), were attributed to the nervous system. This opinion has been held by a long line of exceptional investigators reaching down almost to the present day (Lister, 1854; Bimmermann, 1878; Biedermann, 1892, 1926; Kahn, 1922; Perotti, 1928). To any one familiar with the results of nerve cutting in frogs and in bony fishes no contrast could be sharper. The absence of changes in tints of the skin on cutting cutaneous nerves or more deep-seated autonomic trunks is as characteristic of frogs as its presence is of teleosts. So universal is the absence of color change on cutting, stimulating, or otherwise treating integumentary nerves in frogs, toads, and related forms that one is forced to accept the general conclusion of Hogben and his associates (Hogben and Winton, 1922–1923) that the nerves in these vertebrates are without significance for color changes. Such changes appear to be brought about exclusively by endocrine agencies. So fully supported is this conclusion that the real question today is not nerves versus endocrines or better neurohumors, but which and how many neurohumors are involved in the chromatophore responses of these animals.

That the dark phase of amphibians is due to a

substance from the intermediate lobe of the pituitary gland called by Hogben and Slome (1931) B-substance and by Zondek and Krohn (1932a) intermedin, has been supported by the work of a host of investigators (Smith, 1920; Allen, 1930; Atwell, 1921, 1935, 1937; Giusti and Houssay, 1921; Swingle, 1921; Krogh, 1922; Hogben and Winton, 1922a, 1922b, 1922c, 1923; Hogben, 1924a, 1924b, 1926; Fenn, 1924; Houssay and Giusti, 1929; Charles, 1931; Zieski, 1932; Burns, 1934; Aubrun, 1935a, 1935b; Jores and Caesar, 1935; Abramowitz, 1936b; Osborn, 1936; Atwell and Holley, 1936; Parker and Scatterty, 1937; and many others) and is now generally conceded. There is not the least doubt at present that the single neurohumor, intermedin, is responsible for the dark phase of frogs and probably all other amphibians.

The pale phase of the frog was attributed by Hogben and Winton originally to the disappearance from the animal's blood of the dispersing neurohumor, their B-substance. Hogben, however, and another of his associates, Slome, after an extended study of the color changes in the South African toad *Xenopus*, were led to conclude (Slome and Hogben, 1928, 1929; Hogben and Slome, 1931, 1936) that in addition to intermedin there was in this toad also a concentrating neurohumor actively concerned with the blanching of this form. The chief evidence for this opinion was found in the fact that when the pituitary gland was removed from this toad, it failed to blanch as completely as it did when with its pituitary gland intact it was exposed to a white illuminated environment. The more complete blanching when the gland was still in place indicated, in the opinion of these workers, the production by the toad of a second substance, their W-substance, whose effectiveness was lost with the loss of the gland. The part of the pituitary complex believed by these authors to be the seat of the formation of the W-substance was the pars tuberalis not only in *Xenopus* but also in *Rana fascigula*.

The production of W-substance in the American frog *Rana pipiens* was investigated by Parker and Scatterty (1937). This frog becomes fully blanched after an injection of a small amount of adrenalin, after its blood-vessels have been thoroughly irrigated with Ringer's solution, or after hypophysectomy. The melanophores in such blanched frogs are fully punctate and could not concentrate their pigment further. That they are still normally responsive to chromatic excitants can be shown by injecting a solution of intermedin into such pale frogs whereupon their color cells soon disperse pigment and the frog as a whole darkens. These pale frogs are as a rule slightly paler than normal frogs that have been kept in a white-walled, illuminated vessel. In this respect they are unlike *Xenopus* and they thus fail to yield any evidence in favor of the presence of W-substance. This conclusion is supported by the observation that though blood serum from a dark Rana pipiens when injected into a pale one will darken it, that from a pale one has no influence on the color of a dark individual of the same species.

The conclusion to be drawn from this investigation is that the control of the melanophores in *Rana pipiens* appears to be like that originally ascribed by Hogben to amphibians in general, a pituitary dispersing agent whose absence from the blood allows the melanophores to concentrate their pigment. From this standpoint *Rana pipiens* may be said to be unihumoral as contrasted with *Xenopus* where the conditions appear to be those of a bihumoral animal.

In this connection the condition of frogs blinded by enucleation is not without interest. A frog blinded by the loss of its eyes soon assumes a constant relatively dark tint, but one that is not so dark as that of full pigment dispersion (Parker, Brown, and Odiorne, 1935). It is difficult to explain this state from the neurohumal position except on the assumption of a steady, low discharge of intermedin. Such a discharge apparently goes on whether the blinded frog is in the dark or in the light, but it is not uniform under these two conditions. In the light the frog is a little darker than in darkness, conditions that imply that some organ in its body other than the eye must exert a mild control over the discharge of intermedin, or that the melanophores respond directly to light or its absence.

Amphibians such as *Rana pipiens* are not the only unihumoral vertebrates known. Within recent years two others have been described, namely *Lampetra* among the cyclostomes and *Anolis* among the lizards. *Lampetra*, whose color changes have been studied by Young (1935) is provided on the upper part of its body by melanophores of the usual vertebrate type. Below it is uniformly pale. When individuals were placed in white or black illuminated receptacles no differences could be detected, for in both situations the lampreys remained maximally dark. On further examination, however, they were

found to have a pronounced daily rhythm in color change, becoming pale by night and dark by day. Continuous artificial illumination produced maximal darkening and stopped the daily rhythm, but total darkness allowed the rhythm to persist though diminished in extent. The cutting of spinal nerves was not followed by local changes in the melanophores and it was rightly concluded by Young that these effectors were not under nervous control. The removal of the pituitary complex by a buccal operation or the destruction of its nervous and intermediate lobes reduced the lamprey to a condition of continuous paleness which could be changed temporarily to the dark state only by the injection of mammalian or other vertebrate intermedin. The paleness induced by the loss of the pituitary complex persisted in a lamprey that lived as long as eleven months after the operation. A thorough test of *Lampetra* revealed to Young no means of blanching in this animal except the loss from its blood of the darkening pituitary secretion. He therefore concluded that the color changes in *Lampetra* were dependent entirely upon one neurohumor from the pituitary gland and that the presence or absence of this neurohumor was determined by nervous action. Thus *Lampetra*, like *Rana pipiens*, appears to be a good example of a chromatic vertebrate whose color changes are on a unihumoral basis.

Another example of a chromatic vertebrate with a limited number of neurohumors is the American iguanid *Anolis carolinensis*. The color reactions of this lizard have been studied recently by Kleinholz (1936). On a white illuminated background *Anolis* becomes bright green, on a black one dark brown. In darkness it takes on the green tint. According to Carlton (1903) and von Geldern (1921) the only active chromatophores in the skin of this lizard are the melanophores. It was assumed by these earlier workers and even as late as 1924 by May that these color cells were under direct nervous control. May based his opinion on experimental results from the transplantation of chromatic skin. According to him a piece of skin transplanted from one *Anolis* to another remained green for some two to three weeks. After this period it began to regain its power to change and soon acted in unison with the rest of the animal in its chromatic alterations. This recovery was believed by May and others to be the result of nerve regeneration. Kleinholz demonstrated, however, that in transplants made on the same animal, autotransplants, color changes could be detected on the second or third day after the operation and that when a transplant was made from a hypophysectomized donor to a normal recipient and well fitted to the recipient's body, color changes excited by background differences could be seen in the graft within twelve hours. These changes lagged only a little behind the corresponding ones in the normal skin of the recipient. Such intervals of time as those pointed out by Kleinholz are much too brief to allow of nerve regeneration as an explanation of recovery and they led Kleinholz to suspect that this chromatic recovery was to be associated with renewed vascular connections rather than with nervous changes. Loss of the hypophysis was always followed by permanent pallor (green) which could be temporarily converted into the dark phase (brown) by the injection of pituitary extract. Denervated patches of skin underwent these changes. Electrical stimulation of the mouth of a normal *Anolis* evoked darkening, a color change which failed to appear where this type of stimulation was applied to the same part of a hypophysectomized individual. Hence this form of stimulation is believed to affect the pituitary gland. No special means was discovered by Kleinholz whereby *Anolis* blanched and he finally concluded that this operation (turning green) was due to the disappearance from the circulation of the pituitary intermedin. Thus the ordinary dark (brown) and pale (green) phases of this lizard were shown to be not directly concerned with nerves, but to be dependent upon the presence or absence of a single pituitary neurohumor. Thus so far as its chief color changes are concerned, *Anolis* is unihumoral. Kleinholz, however, also found in the color pattern of this animal a mottled state which he showed was not dependent upon nerves, but which could be excited by the injection of adrenalin and disappeared on the loss of the adrenal glands. Thus an additional neurohumor must be admitted to the chromatic system of *Anolis*, but this element is not necessarily concerned with the ordinary dark and pale changes of this lizard which, as just pointed out, appear to be on a unihumoral basis. Thus *Rana pipiens*, *Lampetra planeri*, and *Anolis carolinensis*, so far as their ordinary pale and dark changes are concerned, seem to be unihumoral. *Anolis*, however, shows evidence of beginning bihumoralism which is apparently true of *Xenopus*. In all these instances direct nerve action is excluded and the color changes in these three forms depend strictly upon hydrohumors.

3. Elasmobranch Fishes

The serious study of the color changes in elasmobranchs began with the work of Lundstrom and Bard (1932) on the smooth dogfish *Mustelus canis*. This fish has well pronounced pale and dark phases dependent upon melanophore activity. When its pituitary gland is removed it becomes permanently pale, as was first shown by Lundstrom and Bard and subsequently confirmed by Parker and Porter (1934). All other elasmobranchs that have been tested in this way show the same peculiarity (*Squalus acanthias*, Parker, 1936a; *Scyllium canicula, S. catulus, Rhina squatina, Raja brachiura, R. clavata, R. maculata, R. microcelatus;* Hogben, 1936; Wykes, 1936; Waring, 1936a, 1936b). An injection of an extract from the pituitary gland into such pale elasmobranchs is always followed by a darkening of their integument. No other organ than the pituitary gland is known to be connected thus with the dark phase of these fishes. If the blood of a dark *Squalus* is injected into a slightly pale one, a dark spot is produced though such blood has no effect on a dark fish. Blood from a pale fish induces no color change when injected into either a dark or a pale individual (Parker, 1936a). What is true of the blood of *Squalus* appears also to be true of that of *Mustelus* (Parker and Porter, 1934). These and other like observations have convinced most investigators that the dark phase of elasmobranchs is excited by a neurohumor produced in the pituitary gland and carried thence by the blood to the responding melanophores.

The pale phase of *Mustelus* was not closely investigated by Lundstrom and Bard (1932) who merely intimated that it was probably due to the absence from the blood of this fish of the dispersing substance intermedin. Parker and Porter (1934) showed, however, that when radiating nerves in the pectoral fin of *Mustelus* were cut, a pale band formed over the denervated area and reached from the cut to the edge of the fin. The initiating cut naturally interfered more or less with the circulation of blood in the part of the fin concerned, but this interference was distinctly local, for, as could be seen under the microscope, it was limited to that part of the band near the cut, the peripheral portion retaining what appeared to be a fully normal blood supply. Hence Hogben's suggestion that such bands are the direct result of vasomotor disturbances fails to apply. Moreover such bands may be excited by the electrical stimulation of nerves without any interference whatever with blood vessels (Parker, 1935a, 1936b). Furthermore when the blood supply to a fin is at a complete standstill in consequence of placing a tourniquet around the base of the fin, a band may still be produced by cutting the nerves (Parker, 1938c). A pale band in a dark fish will in time darken and after it has darkened, it may be revived by recutting the nerves distal to where they were first cut (Parker, 1936b) thus demonstrating that blanching in *Mustelus* is not due to the loss of pituitary secretion, to vasomotor changes, paralysis or the blocking of central impulses, but to the excitation at the cut itself of concentrating nerve fibers which then act directly on the melanophores.

Although pale bands or patches can be produced with great certainty in *Mustelus* by cutting its integumentary nerves, these bands have not been excited in other elasmobranchs subjected to appropriate cuts. In *Squalus acanthias* the cutting of nerves is only rarely followed by the formation of a pale band or a patch and when such areas are produced they are always very faint. In *Raja erinacea* such bands cannot be excited at all and the same seems to be true of *Scyllium* (Young, 1933; Waring, 1936; Wykes, 1936) and of *Raja brachyura* and *R. maculata* (Wykes, 1936). The electrical stimulation of nerves was found by Wykes (1936) to be followed by no color changes in *Raja brachyura, Rhina squatina,* and *Scyllium catulus*. Thus it appears that, so far as is known at present, the formation of well defined, pale bands and patches by nerve cutting is a peculiarity of *Mustelus*.

If *Mustelus* blanches through the action of concentrating chromatic nerves which appear to be absent from other elasmobranchs, how is blanching accomplished in such other forms? In answer to this question two suggestions have been made. Elasmobranchs without concentrating fibers become pale either through the disappearance from their blood of the dispersing pituitary neurohumor already shown to be there under certain circumstances, or they blanch in consequence of the production in their bodies of a concentrating neurohumor such as the W-substance of Hogben.

Hogben (1936) sought for this substance in *Scyllium canicula, S. catulus,* and *Raja brachyura* whose anterior pituitary lobes were suspected of containing it. When this lobe is removed from a dark fish no color change was noted. When it was taken out of a pale fish, the animal became dark even on a white background, as Waring

(1936) also observed. Implantation of an anterior lobe into a pale fish produced negative or relatively slight results. Implantation of such a lobe into a dark fish produced so uncertain a change as to be quite inconclusive. After the removal of the anterior lobe from *Mustelus*, the fish continued to change color normally. But this ability may have depended in part at least on the concentrating nerve-fibers possessed by this dogfish. In *Raja erinacea*, where there are no such nerve-fibers, the loss of the pituitary gland is followed by maximum pallor showing that this gland is not necessary for this extreme reaction. Blood transfers from pale fishes, *Mustelus* (Parker and Porter, 1934) and *Squalus* (1936a), to dark ones produced neither pale spots nor any general change of tint on the part of the recipients. The same was found to be true of transfers of blood carried out on *Raja erinacea* (Parker, 1937b). Thus several elasmobranchs, but especially *Raja erinacea*, give no evidence of a W-substance. Whether further tests will show the presence of this material in those forms from which Hogben and Waring removed the anterior pituitary lobe and thus abolished blanching remains to be determined.

Is it possible that the elasmobranchs other than *Mustelus* blanch by the disappearance of intermedin from their blood? In the elasmobranchs studied by Wykes (1936) *Raja*, *Rhina*, and *Scyllium*, no special tests such as blood transfers were made and consequently no opinion can be formed as to what may be contained in their blood. In *Raja erinacea* the case is different. This fish has been shown to have no concentrating nerve-fibers and its blood when appropriately transferred gives no evidence of containing a concentrating neurohumor. Its only way of blanching seems to be by the loss of the dispersing pituitary neurohumor from its blood. Thus the elasmobranchs in last analysis present a variety of conditions in their chromatophoral system. All, so far as is known, darken under the action of the dispersing pituitary secretion, intermedin. *Raja erinacea* appears to blanch in consequence of the loss of this substance and may therefore be regarded as unihumoral. *Scyllium canicula*, *S. catulus* and *Raja brachyura* may become pale because of a concentrating neurohumor, the W-substance of Hogben, and may therefore be designated as bihumoral species. *Mustelus* appears to be the only elasmobranch thus far known that blanches regularly in consequence of the action of concentrating nerve-fibers.

It is a remarkable fact that the chromatophoral system of the horned toad, *Phrynosoma*, as worked out recently (Parker, 1938a), shows a decided resemblance to that of *Mustelus*. In this reptile the dark phase is dependent primarily upon intermedin and the pale one chiefly upon concentrating nerve-fibers, though these appear to be supplemented in *Phrynosoma* by a hydrohumor, probably adrenalin, of which there is apparently no trace in *Mustelus*.

4. Teleost Fishes

A. *Fundulus heteroclitus* is a small cyprinodont fish from the coastal waters of the Eastern United States. It possesses at least three kinds of chromatophores: melanophores often associated with iridocytes (Foster, 1937), xanthophores, and guanophores (Odiorne, 1933). It may assume a variety of color phases (Connolly, 1925); of these the most conspicuous ranges from a pearly white to a dark steel-gray and depends chiefly upon the melanophores. At 18° to 19° C. *Fundulus*, when transferred from an illuminated white bowl to a black one in full light will darken in a little less than a minute and when transferred in the reverse direction will blanch in somewhat over two minutes (Parker and Brower, 1937). How these responses are accomplished will now be considered.

Since the days of Brücke (1852) it has been known that when a faradic current is applied to the chromatophore tracts or nerves in many vertebrates, these animals blanch through the concentration of pigment in their melanophores. This response is eminently true of *Fundulus*. When such a current is led through the medulla of a dark *Fundulus*, the whole fish quickly loses its deep tint. A similar test on a pale individual is followed by no color change whatsoever. If the current is made to impinge on a bundle of nerves in the tail of a dark *Fundulus*, a pale band is formed from the region of application of the electrodes to the edge of the fin. If a bundle of nerves is cut in the tail of a moderately dark *Fundulus* whereby a still darker band of denervated tissue is produced, the application of a stimulating current to the medulla of the fish will induce its complete blanching except for the denervated band. These and other like tests have long since shown that the pale phase of *Fundulus*, like that in a number of the other lower vertebrates, is under the direct control of concentrating autonomic nerve-fibers, a conclusion that, in its general application, has been admitted by almost

all workers in this field (Brücke, 1852; Sand, 1935; Parker, 1938a).

The dark phase of *Fundulus* is not so easily dealt with. It may be induced in all probability both by dispersing nerve-fibers and by a neurohumor such as the intermedin from the pituitary gland. When a small transverse cut is made in the root of the tail of a pale *Fundulus* so as to sever a few caudal nerves, a dark band begins to appear in about half a minute and will soon be seen to reach from the cut to the edge of the tail (Fig. 1). After five minutes this band will be

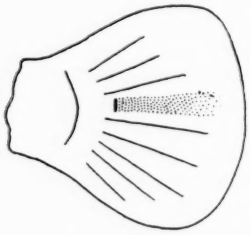

FIGURE 1. Caudal fin of a pale *Fundulus* showing a band of dark melanophores produced by the severance of radial nerves near the root of the tail. Parker, 1934 f, p. 307.

found to be very dark in consequence of the extreme dispersion of pigment in its melanophores. Such bands or dark areas of one form or another have been known for a long time in, for instance, the chameleon and a great variety of teleosts. They have been interpreted as due to the paralysis of the cut nerve-fibers and the consequent lapse of the melanophores into a resting state of expansion. Such a view was held by Brücke (1852) and has been accepted in the main by most investigators down to the present time (Sand, 1935). It may be designated as the paralysis hypothesis of chromatophore reaction (Parker, 1936c).

That this hypothesis is probably incorrect can be shown by a simple experiment on *Fundulus*. A dark caudal band produced in the tail of a pale *Fundulus* (Fig. 1) in the way already described will reach its maximum of coloration in a fraction of an hour or so after which it will begin to fade until in the course of a day or two it will have attained to about the same degree of paleness as that of the rest of the fish. If now a second transverse cut is made slightly distal to the first one, and within the limits of the original band, a second dark band will quickly appear and occupy much of the space originally taken up by the first band (Fig. 2). The production of a second band points to two very significant conclusions. It shows, first, that when chromatophore nerve-fibers are cut they are not at once paralyzed, as had generally been assumed, but that they are capable of renewed activity as judged by the pigment dispersion in the melanophores of the second band. It also shows that the formation of such

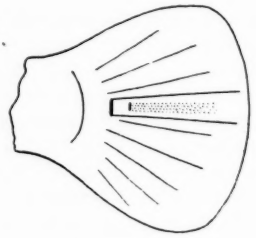

FIGURE 2. A faded caudal band in a pale *Fundulus* within which a new short cut has been made. This cut has induced the formation of a small new band within the large one showing that the severed nerve-fibres of the larger band are still active. Parker, 1934 f, p. 308.

bands is not dependent upon the interruption of central influences, such as tonic or inhibitory impulses, as has recently been suggested by Zoond and Eyre (1934), Zoond and Bokenbam (1935), and Sand (1935), for all such impulses are eliminated by the first cut. The fact that a second cut can be formed shows that this band is produced by some disturbance in the cut itself and not by an agent from a region proximal to the first cut. What the effective disturbance is that gives rise to second bands is not known. That it is not due to the rubbing together of the raw faces of the wound is certain, for a band will form from the distal border of a window cut in the tail where the wound faces cannot possibly rub one another (Fig. 3). Presumably the stimu-

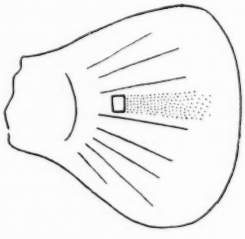

FIGURE 3. A caudal band in a pale *Fundulus* produced by cutting a window-like opening in the tail instead of making merely a slit. Parker, 1934 f, p. 308.

lus for band formation is the action of disintegration products in the cut on the ends of the severed nerve-fibers on the distal face of the cut. But however this may be, the formation of the second band renders highly improbable any hypothesis that has been proposed to account for the formation of caudal bands on the ground of paralysis or the interruption of central impulses.

That the formation of what may be called primary and secondary caudal bands in *Fundulus* as described in the preceding paragraphs is not complicated by the degeneration or regeneration of the melanophore nerve-fibers is seen from the fact that the changes in bands here considered may all occur within the three or four days immediately following the first severance of the chromatophore nerves, whereas degeneration in these nerves is known not to be physiologically significant till five or six days after that time. Regeneration of these fibers begins on about the eighteenth day after the initiation of a primary band and is completed over the whole length of the band on approximately the twenty-fifth day subsequent to the first cut (Parker and Brower, 1933).

That intermedin from the pituitary gland of the fish does not play a necessary part in the formation of the secondary band is evident, for the denervated area on which this band is formed may be fully blanched before the new band is initiated, a condition which would not occur if a physiologically significant amount of intermedin were present in the blood of the pale fish.

If the formation of caudal bands in *Fundulus* is not dependent upon the paralysis of chromatophore nerve-fibers, upon the obstruction of tonic or inhibitory central impulses, upon degeneration or regeneration of nerves, or upon the presence of small amounts of intermedin in the blood, what is it dependent upon? A most likely answer to this question is that the melanophores in such bands as those just described possess dispersing nerve-fibers as well as concentrating fibers and that the act of cutting stimulates these dispersing fibers; in other words that these color cells are doubly innervated. This view was long ago advocated for chromatophores in general by Bert (1875) who failed, however, to advance any very cogent reasons for its acceptance. Aside from what has just been stated, is there any reason for assuming that the melanophores in *Fundulus* are doubly innervated? In answer to this question three kinds of evidence may be considered.

The pharmacological study of the automonic innervation of chromatophores in fishes as carried out by Giersberg (1930) on *Phoxinus* and more especially by Smith (1931a) on *Fundulus* favors the idea of double innervation. Smith in particular used a number of drugs some of which were excitants and others depressants of sympathetic and of parasympathetic nerve-fibers, and concluded, as did Giersberg from his work, that on the whole the results were more favorable to the view of double than of single innervation. A second and more conclusive line of evidence came from Mills' study (1932) of the exact limitations of caudal bands in *Fundulus*. When the edge of a partly blanched band in this fish is scrutinized in detail under conditions in which the rest of the fish is at one time dark and at another pale, individual melanophores are found which respond in an unusual and surprising way. For instance melanophores can be identified which will expand when the fish is on a black background, but which will fail to contract when it is on a white one. Such a condition is difficult to explain except on the assumption that the given melanophore has retained its dispersing nerve-fibers when by the accident of the cut it has been deprived of its concentrating fibers. Clearly single innervation would not suffice to explain this condition. A third line of evidence has been advanced by Abramowitz (1935b, 1936a), who in a study of the regeneration of melanophore nerves in *Fundulus* found somewhat similar conditions at the advancing end of the regenerating nerve. If after the regeneration of the nerve-fibers over a denervated caudal band has got fully under way, the fish is induced to change first pale and then dark. These changes will be registered not only on the fish as a whole, but also on that part of the caudal band in which the nerves have newly formed. Such changes will of course not be seen in the more distal part of the band into which the regenerating fibers have not yet spread. If on a given band the region of transition between the regenerated and the degenerated portions is carefully scrutinized under first the pale phase and then the dark one, the individual melanophores will present highly significant conditions. Some will be found that, like normal melanophores, are capable of full concentration and of full dispersion of pigment; others can be seen that, like fully denervated melanophores, are incapable of either pigment concentration or dispersion; still others will be noticed that can concentrate their pigment, but not disperse it; and finally others will be found that cannot con-

centrate but can disperse their coloring matter. Of these four classes, as Abramowitz has pointed out, the last two seem inexplicable except on the assumption that each melanophore is provided normally with both dispersing and concentrating fibers and that in the first of these two instances the regenerated concentrating fibers have reached the given melanophore in advance of the dispersing fibers and in the second the reverse is true. Such an explanation, and there appears to be no other, implies that normally these melanophores are doubly innervated.

If then the melanophores in *Fundulus* are doubly innervated, the two sets of nerve-fibers, concentrating and dispersing, must of necessity possess a certain amount of physiological distinctness. It is therefore not surprising that one set, the concentrating fibers, should be easily excited electrically and the other, the dispersing fibers, open especially to activation by cutting. It is probable that both sets of fibers are stimulated by both means, but that one is especially receptive to one type of stimulus and the other to the other. It is not to be supposed that these two kinds of stimuli are in any sense exclusive for the two classes of fibers.

Another agent that has been suspected of playing a part in the color changes in *Fundulus* is the melanophore-activating principle of the pituitary gland, intermedin (Zondek and Krohn, 1932a). The possibilities of the pituitary gland in this respect were tested in *Fundulus* as early as 1924 by Desmond who found that after hypophysectomy, this fish was still able to change under appropriate conditions from pale to dark and the reverse. Desmond, therefore, concluded that the melanophore system of *Fundulus* was not influenced by pituitary secretions. This conclusion was in agreement with observations on blood transfers, for the blood of a dark *Fundulus* when injected into a pale one was found to have no observable effect upon the color of the recipient, nor had that of a pale fish on a dark one (Mills, 1932b; Parker, 1934d). Matthews (1933), without knowledge of Desmond's results, also hypophysectomized *Fundulus* and confirmed Desmond's earlier findings. Matthews, however, observed further that when fresh scales from *Fundulus* were immersed in an extract of the pituitary gland of this fish, the melanophores in the scales contracted. This peculiar response led Kleinholz (1935) to experiment further on this subject with the result that he found that the extract from the pituitary gland of *Fundulus*, though ineffective as a means of color change for normal *Fundulus*, would on injection darken *Ameiurus*, *Rana*, and *Anolis*. This extract would also darken a denervated, pale, caudal band in a pale *Fundulus*. Presumably in a fish in this state the intermedin in its blood is so small in amount as to be unable to compete with the concentrating nerve-fibers by which the fish is kept pale, but when by denervation, as in a pale caudal band, these and all other fibers are excluded from acting on the melanophores, the low concentration of intermedin present in the blood becomes effective enough to darken the pale band, though not sufficient to darken any other part of the fish.

This explanation has in fact been confirmed by Abramowitz (1937a) who, recognizing the great sensitiveness of the frog to intermedin, used this animal as a test for small amounts of this agent. He adopted as a standard frog-unit the amount of intermedin in 0.2 cc. fluid which when injected in the dorsal lymph sac of a hypophysectomized frog would maintain the melanophores of the test animal in expansion for a period of three hours. The pituitary gland in a *Fundulus* of ordinary size, 10 cm. long, was found on ordinary extraction to contain approximately four frog-units though upon special treatment 100 such units could be obtained. Further tests showed that the minimal effective dose of intermedin for the denervated tail melanophores in *Fundulus* was 100 times greater than that for the melanophores of the frog. By tests on the frog Abramowitz showed that the blood from a pale *Fundulus* contained intermedin, but it was in such small amounts as to be ineffective in exciting melanophore expansion in that fish. As the blood of even a dark *Fundulus* on injection into a pale fish does not appreciably darken the second fish (Mills, 1932b; Parker, 1934d), it is improbable that under normal conditions intermedin has any real rôle in the color changes of *Fundulus*. This substance is at best only a mild supplement to the action of the dispersing fibers. Except for the remote possibility of a concentrating pituitary neurohumor indicated by Matthews (1933), the agents concerned with the melanophore changes in *Fundulus* appear to be three in all: concentrating nerve-fibers for blanching, and dispersing nerve-fibers, and, possibly, intermedin for darkening.

Fundulus heteroclitus possesses beside melanophores also xanthophores and guanophores. These two types of color cells have been much less studied than the melanophores. The xantho-

phores of *Fundulus* have been worked upon by Fries (1927, 1931), Warren (1932), and Abramowitz (1935a, 1936d). They have been shown by Fries to be innervated and to respond independently of the melanophores and by Abramowitz to possess neurohumors different from those of the black cells. Little is known about the guanophores except that they are present in *Fundulus* and respond to adrenalin by dispersion of pigment as shown by Odiorne. These additional pigment cells, the yellow and the white, add to the already complicated picture of the chromatophore system in *Fundulus*.

B. *Ameirus nebulosus*, the common catfish of the fresh-water ponds and streams of the eastern United States, is another fish that is very satisfactory for the study of color changes. Its range in tint is from a pale greenish yellow to an almost full black. The chromatophores in *Ameiurus* appear to be all of one kind melanophores (Parker, 1934e; Odiorne, 1937). In this respect *Ameiurus* is simpler than *Fundulus*. The mechanism of the color changes in *Ameiurus*, as the following account will show, is more complex than that in *Fundulus*. *Ameiurus* is slow in color responses as compared with *Fundulus* and requires hours to accomplish changes that are made by *Fundulus* in minutes. Thus a dark *Ameiurus* will blanch on a white background in from twenty-four to thirty-six hours and will darken in a black one in from fifteen to twenty-four hours (Parker, 1934e). The periods for these changes as given by Abramowitz (1936c) namely for blanching less than two hours and for darkening less than one hour, are shorter than those just noted probably because this worker used somewhat different turning points in his tests. In consequence of the slowness of the color changes in *Ameiurus* as compared with *Fundulus* some experimental procedures easily carried out on the latter are impossible on the former.

Parker (1934e) was unable on applying electrical stimulation to the medulla of *Ameiurus* to elicit blanching as in *Fundulus*, but Abramowitz (1936c) was more successful in that he induced a paling of the tail of *Ameiurus* on stimulating electrically the base of the spinal column. Blanching induced in this way had no effect on dark caudal bands. Thus there is good evidence in *Ameiurus* for the presence of concentrating nerve-fibers. By cutting radial nerves in the tail of a pale catfish well marked dark caudal bands may be produced. These are relatively persistent and may not wholly disappear in a pale fish for a week or more. Their proximal portions blanch earlier than their distal ones. When a second cut is made in the blanched portion of such a caudal band, a secondary band is formed though not as conspicuously as in *Fundulus*. Nevertheless in *Ameiurus* such secondary bands are easily demonstrable and fully support the assumption that dispersing nerve-fibers are present in this fish as in *Fundulus*. This conclusion was confirmed by Abramowitz (1935b) in his study of the regeneration of chromatophore nerves in *Ameiurus*.

The pituitary gland in *Ameiurus* is a very efficient organ for color change as contrasted with the corresponding structure in *Fundulus*. When the pituitary gland is removed from an *Ameiurus*, the fish will still change pale or dark under the influence of its two sets of autonomic nerves, but a pale caudal band in such a fish will persist for some time after the fish has become dark, a condition which does not prevail in a fish normal in respect to its pituitary gland. Here the intermedin from the gland darkens the pale band as it does the rest of the fish whereas in the absence of the gland no such darkening occurs. As might be expected the blood from a dark normal *Ameiurus* will induce a dark spot when injected into a pale individual, whereas that from a hypophysectomized *Ameiurus*, even though the animal is dark, has no effect upon the color of the recipient. The dispersing nerve-fibers, unlike the pituitary gland, produce no blood-soluble agent.

In addition to intermedin *Ameiurus* has been suspected of possessing a concentrating neurohumor whose properties resemble those of adrenalin. Abramowitz (1936c) noticed that from two to five minutes after a catfish had been much disturbed by movements directed toward its capture, it would often blanch generally. This change did not apply immediately to dark caudal bands that it might possess. Such bands, however, would remain dark for only some five to ten minutes after the rest of the fish had blanched when they would then also blanch. The fish would then gradually darken followed after an interval of time by the bands. This whole reaction has a striking resemblance to the excitement pallor which has been described in lizards by Redfield (1918) and by Hogben and Mirvish (1928a, 1928b) and which was attributed by Redfield to adrenalin. It is natural that Abramowitz should conclude that the agency responsible for such pallor may be adrenalin or a substance

like it in effect. How much such an agent may have to do with the habitual color changes in *Ameiurus* is still to be ascertained.

The melanopore system of *Ameiurus* looked on as a whole is activated by a greater number of agents than is that in *Fundulus*. In addition to concentrating and dispersing nerve-fibers *Ameiurus* possesses a well-developed pituitary control and probably some form of concentrating neurohumor whose action resembles that of adrenalin. Of these several agents it was early believed that the dispersing nerves were probably the most efficient (Parker, 1934e), but it has since been shown with reason that intermedin is in all likelihood the most effective factor (Abramowitz, 1936c; Veil, 1937; Osborn, 1938).

From the standpoint of the present discussion *Fundulus* and *Ameiurus* appear to have been more fully studied than any other teleosts. Veil (1935), however, has advanced much evidence that in *Cyprinus* and *Carassius* in addition to nerves, the pituitary secretions play an important part in color changes. A few observations looking toward the neurohumoral interpretation of color changes have been recorded for the squirrel fish, *Holocentrus ascensionis*, an inhabitant of the subtropical waters of Bermuda. This fish changes from red to white and back again with great rapidity (Smith and Smith, 1935). The averages from a number of time records for these changes at 19° C. are red to white about nineteen seconds and white to red a little over six seconds. These vary rapid changes depend upon the contraction and expansion of erythrophores which give evidence of being doubly innervated. Apparently no other agents have a part in these color changes, for, though the fishes blanch to injected adrenalin and redden to pituitrin, they are not influenced by blood transfers nor by injections of extracts from their own pituitary glands (Parker, 1937c). In these respects *Holocentrus* seems to be much like *Fundulus* was believed to be when its color changes were first described. One important fact that these rather scanty records from *Holocentrus* disclose is that though this fish is unusually rapid in its color changes, it nevertheless employs apparently much the same chromatophore mechanism as the more slowly responding species do. It is very desirable that fuller and more extended studies be carried out on this and other rapidly responding teleosts.

The chromatophore systems in the few teleosts which have thus far been studied from the neurohumoral standpoint appear to be far from uniform. Intermedin was not identified in *Holocentrus*, is present in *Fundulus* only in insignificant amounts, but is an active agent in *Cyprinus* and *Carassius* and especially in *Ameiurus*. Adrenalin or an adrenalin-like neurohumor may occur in *Ameiurus*. Nerves play a direct part in *Cyprinus* and *Carassius* and in *Fundulus*, *Ameiurus*, and *Holocentrus* these are known to be of two kinds, concentrating and dispersing.

5. *Lipohumors*

It was stated in the concluding paragraph of the last section that nerves play a direct part in the blanching and darkening of certain teleosts, particularly of *Fundulus* and of *Ameiurus*, and in the blanching of the elasmobranch *Mustelus*. Is such direct nerve action radically different from the humoral control of color cells, as has been assumed by Hogben, or are both types of control, nervous and humoral, examples of one kind of activation? This question can be approached best by a further inquiry into the nature of the integumentary bands and patches produced by denervation. Such bands and patches were observed and studied in the chameleon as long ago as 1852 by Brücke, but it remained for Pouchet (1876) to point out that in fishes at least darkened patches thus produced were not permanent, but became in course of time as pale as the rest of the animal. Von Frisch (1911a) also noted that the contrast between the denervated dark area and the pale general surface in the minnow may vanish by the blanching of the dark area in from twelve to fourteen days. This observation was confirmed by Smith (1931b) except in so far as the time was concerned. According to Smith the blanching of a dark area in the minnow may be accomplished in the course of several days instead of weeks. Smith further noticed that the band or spot on the minnow became pale only if the fish was kept on a white, illuminated background. On a black one the area remained dark as the rest of the fish did. The blanching of dark areas was also observed by Zoond and Eyre (1934) in the chameleon though in this animal the change required six to eight weeks. It may well be that these several instances, all examples of blanching, may depend in different cases upon different factors and thus call for separate explanations, but, however this may be, the fact is clearly recognized by all who have worked upon *Fundulus* that the dark caudal bands produced by incisions made in the tail of this fish blanch in the course of about two days if the creature

is kept on an illuminated white background. In its time relations this instance is like that of the minnow as described by Smith and not like the chameleon. *Fundulus* moreover resembles the minnow in that its caudal bands fail to blanch when the fish is kept continuously dark in tint.

It was first shown by Mills (1932b) that when a caudal band in *Fundulus* begins to fade, it does so not uniformly throughout its whole breadth, but first on its periphery. Thus the edges of the band are lost earlier than the axis, that is, the melanophores on the edges concentrate their pigment before those in the axis do. This was subsequently demonstrated by Parker (1935c) who with the aid of Abramowitz photographed from hour to hour the same area in a fading caudal band of *Fundulus*. It can be seen by comparing Figure 4 which was taken from a caudal band a quarter of an hour after it had been formed with Figure 5 from the same place in the same band six and a quarter hours after the initiating cut had been made. The concentration of pigment in the peripheral melanophores in Figure 4 is noticeable as compared with what is to be seen in these cells in Figure 5.

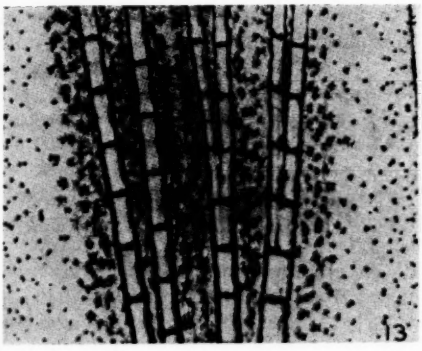

FIGURE 4. A microphotograph of a part of a caudal band in a living *Fundulus* 15 minutes after the cut had been made by which the band was initiated. The band is formed in the region of four fin-rays which are branches from a single ray at the base of the tail (below in the figure). The melanophores composing the band are fully expanded as contrasted with those of the rest of the tail which are, in most instances, fully contracted (right and left of the band in the figure). Parker, 1935 c, pl. 3, fig. 13.

If a small amount of adrenalin is injected into a *Fundulus* with a well marked caudal band, the band will blanch uniformly over its whole width. This is what would be expected from an agent carried in the blood and lymph and thus brought to act on the band over its whole inner surface. The natural blanching of a caudal band does not take place in this way, but, as just indicated, proceeds from the edges of the band toward its axis. It must therefore depend upon some agent that comes from the adjoining pale field, an agent probably the same that has induced the general paleness of that field and that has worked slowly from the periphery into the band.

If this view of the normal blanching of a caudal band is correct, such bands should require different lengths of time to become pale depending upon their width and this seems to be true. Thus a band one millimeter wide in the tail of *Fundulus* will blanch fully in about one day, whereas one two millimeters wide will require for the same change some two days (Parker, 1934a). Abramowitz (1936c) has made similar measurements on *Ameiurus* and has recorded that a band of the width of a single ray will blanch in two days, one of two rays in five days, and finally one of three rays in ten days. All these observations support the conclusion that the blanching of a dark caudal band in a pale fish is due to the invasion of the band laterally by an agent from the adjacent pale field. This agent is unlike adrenalin in that it gives no evidence of being soluble in blood or other watery fluids. It spreads, however, through the tissues and presumably takes its course by way of their lipoid components. It is believed to

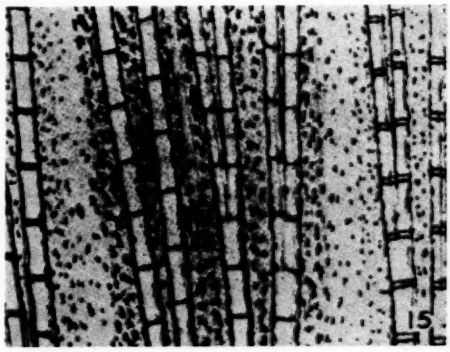

FIGURE 5. A microphotograph of the same part of the caudal band in a living *Fundulus* as that shown in Figure 4 but taken six and a quarter hours after the initiating cut had been made. The greater concentration of the pigment in the peripheral melanophores of the band as compared with that in the axial melanophores can be clearly seen. Parker, 1935 c, pl. 3, fig. 15.

be oil-soluble and to originate from the terminals of the concentrating, autonomic nerve-fibers and to affect not only the melanophores with which these fibers are immediately associated, but to spread to and affect the denervated melanophores of the caudal band itself. The rate at which such bands blanch would then be a measure of this spread, roughly half a millimeter a day in *Fundulus*.

After a caudal band in a pale *Fundulus* has blanched, the fish may be darkened by being placed on a black background with the result that a pale band will be left in the tail of a dark fish.

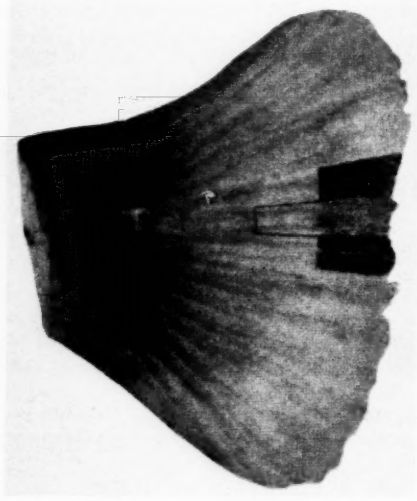

FIGURE 6. Diagram of a caudal fin of a hypophysectomized catfish, *Ameiurus*, with a faded caudal band between two newly cut dark half-bands. The half of the faded band not flanked by the new dark bands has remained pale; the half flanked by the dark bands has darkened slightly. Parker, 1934 e, pl. 1, fig. 5.

In course of time such a band will darken if the fish is kept dark just as a dark band will blanch on a fish kept pale. All such changes in bands are accomplished with a certain amount of lag as compared with the change in the tint of the fish as a whole. Such a lag is quite consistent with the idea of the invasion of the band by a neurohumor from the adjoining fields whereby the change in the band is believed to be produced (Parker, 1933a, 1933b, 1934a, 1935d).

Almost everything that has been mentioned concerning the caudal bands in *Fundulus* may be duplicated for the caudal bands of *Ameiurus* (Parker, 1934e; Abramowitz, 1936c). In this fish in addition an interesting experimental test of the influence of dark lateral fields on a pale band can be made in the following way. If an *Ameiurus* with a dark caudal band is placed on a white, illuminated background, the dark band will blanch in a few days. If now two new dark bands are made by cutting the fin rays adjacent to that of the blanched band, and the cuts of the new bands are made not near the root of the tail as that for the blanched band was, but about half way out on the rays toward the tip of the tail, the result will be a central pale band whose distal half will be abutted laterally by dark bands. The proximal half of the pale band will be surrounded only by the pale portion of the tail. Under such circumstances the distal half of the pale central band which is flanked by the dark half-bands will be seen to darken slowly, whereas the proximal half will remain pale (Fig. 6). This experiment shows quite clearly how adjacent dark areas may bring about a dispersion of pigment in a pale area, a change easily understood from the standpoint of an invading dispersing neurohumor.[1]

All these observations on *Fundulus* and on *Ameiurus* when taken together support the conclusion that the changes in tint of caudal bands in these fishes are due to the invasion of the bands, be they pale or dark, by agents from the opposing adjacent fields and that these agents are dispersing or concentrating neurohumors produced by the appropriate sets of nerve-fibers in these fields. Such neurohumors, insoluble in water, are believed to spread from their regions of origin through the lipoid constituents of the tissues to the melanophores in the denervated bands. In these respects *Fundulus* and *Ameiurus* seem to be essentially alike.

The dogfish, *Mustelus canis*, has a type of melanophore innervation quite unlike that of *Fundulus* and *Ameiurus*. In *Mustelus* the normal darkening of the fish is not dependent upon the direct action of nerves, but upon intermedin. Its blanching, however, involves concentrating nerve-fibers, a state of affairs which, as already noted, appears to be peculiar to *Mustelus* as compared with the other elasmobranchs thus far investigated. When integumentary nerves in

[1] Since the preparation of the present paper Matsushita (1938) has published a study of the color changes in the Japanese catfish *Parasilurus* in which he has repeated this experiment and devised several others to the same end all of which confirm the idea of lateral neurohumoral transmission.

Mustelus are cut, as for instance in the pectoral fin, a pale band is formed instead of a dark one. This pale band may be excited electrically (Parker, 1935a) as well as by cutting. After it has faded it may be revived by recutting (Parker, 1936b). When intermedin is absent from the blood or low in amount, the edges of such a band are sharp and straight; when this agent is present in functional quantities in the blood, the band commonly has irregular outlines or may be in large part obliterated (Parker, 1937d). As the pale band is strictly limited to the denervated area and never spreads noticeably into the dark surrounding fields, but may be encroached upon by the dispersing effects of intermedin, it is concluded that it may result, as with the pale band in *Fundulus* and *Ameiurus*, from a lipoid-soluble neurohumor produced by the terminals of the concentrating nerve-fibers.

If the concentration of melanophore pigment in *Mustelus* and its dispersion as well as its concentration in *Fundulus* and in *Ameiurus* are dependent upon neurohumors insoluble in aqueous media but soluble in oily materials, it ought to be possible to extract such neurohumors by the appropriate treatment of fish skins. This has been attempted and with reasonable success in *Mustelus* and in *Ameiurus*.

The phase of *Mustelus* that is suspected of being associated with an oil-soluble neurohumor is the pale one and the parts that show this phase best are the fins. Smooth dogfishes were therefore put in a white-walled illuminated tank and after a few days, when they had become fully blanched, they were killed and their fins removed. It was a matter of good fortune that in the preparation of the fins the cutting of nerves intensified the paleness rather than the reverse. The fins immediately after their removal were ground to a pulp and the pulp from the fins of one ordinary dogfish was then thoroughly mixed with about two cubic centimeters of Italian olive oil. This mixture was further ground by hand for about half an hour in a rough porcelain mortar till it reached the consistency of a thick paste and then it was set aside to undergo extraction. In most instances it was sterilized by heat before it was extracted. Its extraction was carried on at the low temperature of an ordinary ice box. After the paste had stood some fifteen hours, it was mixed with its own volume of sterile seawater and the thick liquid that resulted was set aside to allow the oil to rise to the top. In this way there was collected a crude water-and-oil emulsion which after having been roughly filtered through sterile cheesecloth, was vigorously agitated and injected subcutaneously in appropriate amount into a dark dogfish. Very soon after the injection had been made there commonly appeared on the skin of the dogfish and a little in front of the point of insertion a few small pale spots which, however, soon disappeared. As these spots were to be seen when small amounts of indifferent fluids were injected as controls, they were regarded as of purely operative significance. In from one to two days after the injection relatively large pale areas made their appearance in the skin immediately over the region into which the fin extract had been introduced (Fig. 7). These

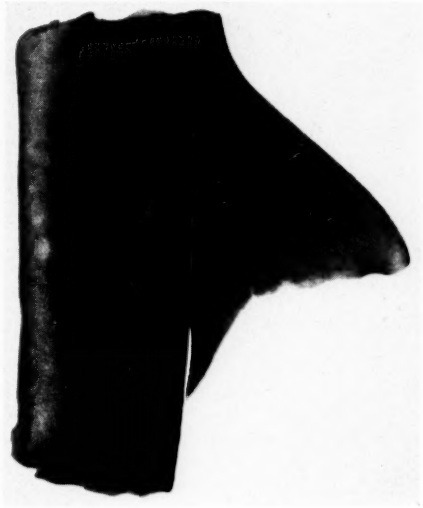

FIGURE 7. Left side of the trunk of a smooth dogfish, *Mustelus*, in the region of the anterior dorsal fin showing a pale spot due to an injection of 0.5 cc. of an emulsion of olive-oil extract from blanched fins in seawater made a little over a day previously. Parker, 1935 b, p. 840, fig. 1A.

large areas were very persistent and, as could be shown under a low power of the microscope, they were produced by the concentration of melanophore pigment. That the pale skin included in these spots was essentially normal was demonstrated by the injection of pituitrin into a fish with such a spot. Shortly after an injection of this reagent had been made, particularly if the region of injection was close to the pale spot, it disappeared by the darkening of its melano-

phores only to return after a few hours as the effect of the pituitrin wore off.

These large pale spots were not produced by injections of seawater, oil, oil extracts of dark fins or of muscle, seawater extracts of pale fins, or defibrinated blood from pale or dark fishes (Fig. 8). They were produced from oil extracts,

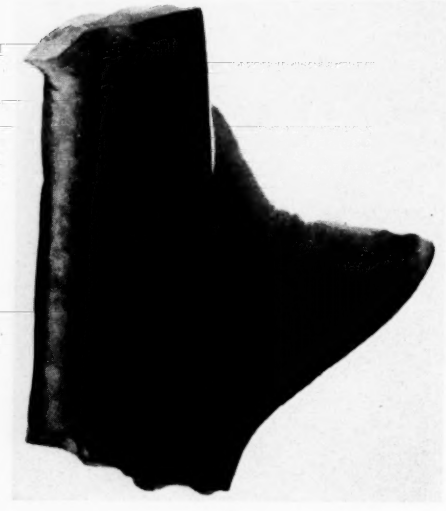

FIGURE 8. Right side of the same dogfish as is illustrated in Figure 7, showing no change of color after the injection of 0.5 cc. of an emulsion of pure olive-oil in seawater. Parker, 1935 b, p. 840, fig. 1B.

sterilized or not sterilized, of pale fins and from cold ether extracts and Soxhlet ether extracts from the same. These various tests led to the conclusion that the induced pale areas in *Mustelus* are due to the action of some substance that can be extracted from the pale fins of this fish by olive oil or ether. The few known properties of this substance aside from its solubility in olive oil and ether and its insolubility in water are its resistance to dry heat up to 110° C., to treatment with two per cent sodium hydroxide and with two per cent hydrochloric acid (Parker, 1937a). This substance may be extracted from fins that have been kept in a dry state in the laboratory for a year (Parker, 1938b). It must therefore be a relatively stable material, such for instance as one of the sterols.

The only other fish that has been examined for the possible presence of oil-soluble neurohumors is the catfish *Ameiurus*. In this fish the dark phase is the one favorable for study. Extracts of the fins and skins of dark catfishes were prepared as in the case of pale dogfishes, and the final extract was injected subcutaneously into pale catfishes. This operation was followed in a little less than an hour by the formation of dark splotches on these fishes (Fig. 9). Such splotches, which were caused by the dispersion of pigment in the melanophores of the region concerned, disappeared spontaneously after a few days. When they were first formed, they could be temporarily obliterated by an injection of adrenalin. Extraction of the skin of *Ameiurus* by ether, hot or cold, yielded residues that were slightly active in darkening the skin, but they were by no means so effective as were the ether extracts from *Mustelus*. However, the evidence from the catfish supports the view that in *Ameiurus* a dispersing neurohumor is present which in its solubility in oil and in its insolubility in water resembles the concentrating neurohumor from *Mustelus*.

From what has been stated in the preceding discussion, it is evident that in addition to those neurohumors, such as intermedin and adrenalin, that are soluble in water, there are others insoluble in aqueous media, but soluble in oil, ether and

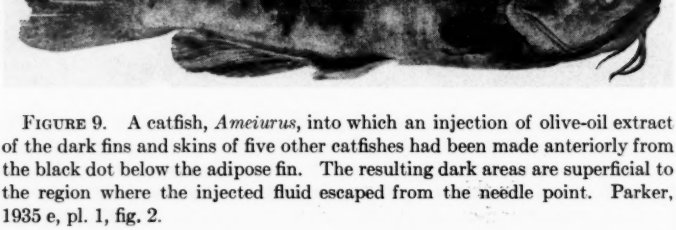

FIGURE 9. A catfish, *Ameiurus*, into which an injection of olive-oil extract of the dark fins and skins of five other catfishes had been made anteriorly from the black dot below the adipose fin. The resulting dark areas are superficial to the region where the injected fluid escaped from the needle point. Parker, 1935 e, pl. 1, fig. 2.

the like. These two classed may be designated conveniently as hydrohumors when they are soluble in water and lipohumors when they are soluble in lipoids, fats, or fat solvents. Lipohumors are from their nature essentially stationary in the animal body and can at best diffuse only slowly from place to place as compared with hydrohumors which may be carried rapidly in the blood and lymph from one part of the body to another. Lipohumors therefore have not attracted the attention that hydrohumors have though the significance of the lipohumor in animal economy may be far from small. The parts played by the lipohumors in the nervous excitation of melanophores show that the distinction often drawn between the nervous and the humoral activation of these color cells is unimportant. In both types of excitation neurohumors are involved. The chief difference is that in hydrohumors the substance comes usually from a remote source, in lipohumors from one close to the color cell.

IV. Neurohumors

It must be evident from what has been stated in the preceding section that the activation of vertebrate chromatophores is dependent upon several neurohumors. First among these is intermedin which appears to play a part in the color changes of all chromatic vertebrates whose pituitary secretions have thus far been examined. This range includes representatives from the cyclostomes to the lizards. Even in *Fundulus* where the influence of intermedin seems to be at its lowest ebb, its effects on the melanophores are slight rather because of the resistance of these color cells than in consequence of any insufficiency in the secretion. Intermedin like many other internal secretions probably has other functions than that of controlling pigment cells, a belief supported by the fact that it is an abundant product in birds and mammals where active chromatophores do not occur. Intermedin is the material which Hogben and his associates have designated in their discussion of vertebrate color changes as the B-substance. Throughout the vertebrates it is the usual means of dispersing melanophore pigment.

A second significant vertebrate neurohumor for color changes is adrenalin. This is by no means so commonly present nor so uniform in action as intermedin. On injection it induces almost invariably a concentration of melanophore pigment whereby the animal blanches. Kleinholz (1936) has pointed out, however, that in *Anolis* adrenalin not only causes a generalized pallor but also induces in this lizard certain dark patches and mottlings. It was shown by Bigney (1919) that when adrenalin is injected into a frog, the integumentary melanophores concentrate their pigment, but the retinal ones disperse theirs. Thus in the melanophores of the same animal adrenalin induces opposite effects. Apparently adrenalin plays a part in the normal color changes of certain lizards such as *Phrynosoma* (Redfield, 1918; Parker, 1938a), and *Anolis* (Kleinholz, 1936b), and in the catfish *Ameiurus* (Abramowitz, 1936c) in all of which it is associated with what Hogben and Mirvish (1928a, 1928b) called excitement pallor. To what extent it may be identified in other chromatic vertebrates remains to be discovered. Its usual source is the medulla of the adrenal glands or other similar but less specialized vertebrate tissues. Closely related to it, if not identical with it, is the neurohumor sympathin (Cannon and Rosenblueth, 1935) as produced by the sympathetic terminals in mammals. Too little is known of the W-substance of Hogben to relate it to adrenalin. Its physiological effect on melanophores is much the same as that of adrenalin, but its assumed origin from the pars tuberalis of the pituitary complex (Hogben and Slome, 1931, 1936; Hogben, 1936) is not especially suggestive of a relation to adrenalin. It may resemble adrenalin only in so far as the similarity of its melanophore response is concerned. Although adrenalin may stimulate the nervous mechanism of the chromatophore system, it certainly also acts directly on the melanophores themselves for, after these color cells have been denervated, they continue to respond to this neurohumor (Parker, 1934b).

In addition to the two neurohumors, intermedin and adrenalin, certain fishes at least appear to possess oil-soluble neurohumors, lipohumors, which are believed to emanate from the controlling autonomic nerve-terminals. One of these, as already noted, is concerned with the blanching of the dogfish *Mustelus canis* and may be provisionally designated as lipohumor A (Parker, 1935b). What appears to be another is effective in darkening the catfish *Ameiurus nebulosus* (Parker, 1935e) and might be set down as lipohumor B were it not for the fact that the crude product containing lipohumor A from the dogfish when injected into *Ameiurus* darkens instead of blanches this fish. Thus the blanching agent of *Mustelus* acts in the reverse way on the

melanophores of *Ameiurus* (Parker, 1938b). If this blanching lipohumor from *Mustelus* is the same as the darkening one from *Ameiurus*, then there must still be another lipohumor in *Ameiurus* to account for its blanching. That one neurohumor such as A can concentrate the melanophore pigment in *Mustelus* and disperse it in *Ameiurus* is not without precedent, for, as Carlson (1935) was first to point out, the crustacean eye stalk neurohumor concentrates the pigment in the dark chromatophores of such shrimps as *Palaemonetes*, but disperses it in the crab *Uca*. These rather remarkable reversed reactions can be accepted, however, only provisionally, for they are all based upon reactions to crude extracts. After these extracts have been studied further, it is entirely possible that what has been assumed to be a single agent may prove to be more than one and thus give ground for other assumptions than those advanced at present.

This brief account of the vertebrate neurohumors, intermedin, adrenalin, and the one or more lipohumors is based almost exclusively on the reactions of melanophores. But a number of vertebrates possess other chromatophores than black ones such, for instance, as xanthophores, erythrophores, guanophores, and the like. Are these additional color cells controlled by the same neurohumors as the melanophores are or do they possess activating hormones peculiar to themselves? This question cannot be answered with any fullness because of lack of information. The responses of xanthophores in *Fundulus*, however, as worked out by Fries (1931) and by Abramowitz (1936d), show that the activators of these cells must be distinct from those of the melanophores. Thus on a black background both xanthophores and melanophores are expanded, on a white one both are contracted, on a blue one xanthophores are contracted and melanophores are expanded, and on a yellow one xanthophores are expanded and melanophores contracted. Thus expanded xanthophores may accompany expanded or contracted melanophores and the same is true of contracted yellow cells. That the xanthophores react independently of the melanophores appears to be probable. If they are thus independent, it would be necessary to assume separate sets of neurohumors, one for the melanophores and another for the xanthophores. Whether or not other types of chromatophores such as the erythrophores and guanophores possess their own systems of neurohumors is still to be ascertained, but the analysis of this problem has already gone far enough to show that the list of the vertebrate chromatophoral neurohumors is one of increasing numbers rather than the reverse.

The number of chromatophoral neurohumors in crustaceans has already been discussed at some length. Judged from the normal color responses of certain shrimps as worked out by Brown (1935) there appears to be ground for the assumption of several neurohumors, a view which Abramowitz has designated as the multiple theory of chromatophoral activators. Judged from the simplicity of the sinus gland from which the one or more of these neurohumors appear to proceed and from the highly differentiated condition of the chromatophores in different crustaceans the diversity of responses has been placed by Abramowitz (1937b) with the effectors rather than with the exciting agent. In fact Abramowitz assumes the possibility of a single neurohumor to account for the chromatophoral changes in crustaceans. This view, the unitary theory, is not supported by what is known for the chromatophoral system of the vertebrates nor has it been worked out in detail by Abramowitz for the crustaceans. Its possibilities must, however, be admitted, though the probability of its correctness seems distinctly remote.

An interesting unifying factor in the whole system of chromatophoral neurohumors is seen in the relation of these agents from one species of animal to the chromatophores of another species particularly where the species tested are from different phyla as, for instance, arthropods and vertebrates. It was shown early by Hogben and Winton (1922a) that intermedin from the ox was as fully effective in the melanophore responses of the frog as its own intermedin was. Koller (1928) also demonstrated that extracts from the eyestalks of several species of *Leander* and from *Crangon* would stimulate the chromatophores in species other than that from which the extract had been made. These observations were extended and confirmed by Koller and Meyer (1930), Kropp and Perkins (1933), Carlson (1935), Kleinholz (1937) and Hanström (1935, 1937). Koller and Meyer (1930) also showed that not only would the extract from one species of crustacean induce color changes in another, but that extracts from the eyestalks of *Crangon* and of *Praunus* would contract the melanophores of the fishes *Gobius* and *Pleuronectes*. These interphylar reactions were extended by the work of Meyer (1930, 1931) and of Kropp and Perkins (1933b)

and Perkins and Kropp (1932), who made the interesting discovery that the crustacean eyestalk extract, which blanched the shrimps from which it was taken, darkened the skin of frog tadpoles. This darkening of the skin by crustacean eyestalk extract was observed in the elasmobranch *Mustelus*, the teleost *Ameiurus*, the common American frog, and the Carolina lizard *Anolis* by Abramowitz (1936e) who also noted an expansion of the erythrophores in the teleost *Chrosomus* by the same agent. Abramowitz further pointed out that intermedin when injected into a pale fiddler crab (*Uca*) induced a dispersion of its black pigment as its own eyestalk extract did. This observation confirmed the earlier one of Böttger (1937) who showed that intermedin could darken *Crangon*. Thus it was demonstrated that not only crustacean neurohumors could activate vertebrate melanophores, but that vertebrate intermedin could excite crustacean chromatophores. Because of the similarity of chromatophore responses to intermedin and to the eyestalk extract Abramowitz (1936b, 1937b) was led to speculate on the possible identity of intermedin and the eyestalk neurohumor.

Having described briefly the neurohumors that are known to be associated with chromatophores, it may not be inappropriate to offer a general definition for this class of substances. A neurohumor may be said to be a hormone produced by nervous tissue or by glands appended to that tissue and serving as an activator or an inhibitor of other nervous tissue or its effectors. Neurohumors as thus defined include not only the substances discussed in this communication, but other agents such as acetylcholin about whose exact relation to chromatophores there appears to be some uncertainty (Wunder, 1931; Parker, 1931, 1934c; Smith and Smith, 1934; Bogdanovich, 1937; Beauvallet, 1938; Chin, 1939).

It does not include such hormones as the original one, secretin, nor those included under the general heads of androgens and oestrogens. The inclusion of intermedin under neurohumors may arouse some comment because of the embryonic source of the part of the pituitary gland concerned with the production of this substance, but such comment seems unnecessary, for the definition of a neurohumor as just given is avowedly a physiological one and not a morphological one. Abramowitz has implied (1936c, 1937c) that the term neurohumor is limited to those hormones that emanate from nerve terminals as do the lipohumors herein described, but this implication was due apparently to a misunderstanding for which a lack of clearness in the writer's papers was probably responsible.

The term neurohumor (Fredericq, 1927) is synonymous with the somewhat less concise one of neurohormone (Huxley, 1935). It is also an equivalent of the common designation chemical activator. This last term implies that such agents act chemically on the receptive cells. That their action is not necessarily chemical, but may be physical in character has already been intimated by Abramowitz and Abramowitz (1938). From this standpoint the term chemical activator seems to imply too much. Nor is there any part of it that relates it to nervous activity especially. In derivation chemical activator is rather the equivalent of the simple term hormone. For these reasons this term seems less desirable than the other two of which neurohumor is slightly the more concise.

It has been found convenient to divide neurohumors into two classes (Parker, 1935b) hydrohumors or those soluble in water and consequently such as may be carried in the circulating fluids of animals, blood, lymph, and the like, and lipohumors of those soluble in fatty and oily materials and that spread probably exclusively by diffusion from cell to cell. In consequence of their solubility in water hydrohumors are commonly capable of exerting almost at once an influence over the whole of a given animal. Lipohumors on the other hand spread very slowly and are chiefly local in their action.

These various substances are the kind of agent presupposed by the neurohumoral hypothesis (Parker, 1932), a conception according to which all the interrelations between receptor cells and neurones, between one set of neurones and another, and between neurones and their appended effectors are by means of specific substances, the neurohumors, rather than by purely electrical steps. So far as the relations of crustacean and vertebrate chromatophore nerves to their end-organs are concerned, there seems to be abundant substantiation of this hypothesis.

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